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# Influence of mycorrhizal strains on height and diameter of *Glycine max* (L.) Merr.

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## Abstract

**Objective**: To evaluate the effectiveness of the application of three strains of arbuscular mycorrhizal fungi (AMF) on the morphophysiological development of *Glycine max* (L.) Merr. cultivar Incasoy 27 in a typical reddish Brown Fersiallitic soil in the Amancio Rodriguez municipality, Las Tunas province, Cuba.

**Materials and Methods**: On a typical reddish Brown Fersiallitic soil, an experiment was carried out under field conditions. The research was developed in the period from September to December, 2020. A randomized block design with five treatments and four replicas was used to set up the experiment. The treatments were: absolute control, NPK and mycorrhizal strains INCAM 4, INCAM 11 and INCAM 2. Plant height and stem diameter were evaluated during the vegetative cycle of the crop.

**Results**: There were significant differences between treatments for the variable stem diameter. The *G. max* plants with the highest diameters corresponded to the treatments with NPK fertilization and with the mycorrhizal strain INCAM 11, with no significant differences between them, and with average diameters above 4 cm. The highest confidence intervals between the comparisons of the treatments appeared when comparing the control with regards to the treatment INCAM 11. For the variable stem diameter, the confidence intervals at 60 days after germination (DAG) for each of the treatments widened with respect to the intervals in the evaluations carried out at 30 DAG. This showed increased phenotypic variability of that trait in the plants of each of the treatments at that crop age.

**Conclusions**: The application of arbuscular mycorrhizogenic fungal strains positively influenced the morphophysiological indicators evaluated in *G. max*, which allows to decrease the use of mineral fertilizers in this crop.

**Keywords**: *Glycine max*, inoculation, mycorrhizae

## Introduction

*Glycine max* (L.) Merr. has been imported from Brazil, Argentina and Asian countries in the last 10 years, which forces to allocate substantial resources to acquire the grain, which is an important component in the intensive production of poultry and swine meat, milk production, yogurt, oil and other foodstuffs (D'Angelo *et al*., 2019). This grain is among the most important grains in the world due to its high protein and fat content.

Natural inputs such as organic manures, composts, biosolids, arbuscular mycorrhizal fungi (AMF) and rhizobacteria are an alternative for biological fertilization. Several studies report that they can improve nutrient absorption in the rhizosphere, produce plant hormones, improve soil physical properties, favor the biodegradation of substances, recycle nutrients, favor microbial synergies, among others (Ceiro-Catasú *et al*., 2023).

AMF have different symbiotic activities, among them mycelium growth, increase of root exploration capacity and, consequently, decrease of the effects of abiotic conditions adverse for the crop, such as salinity and phosphorus immobility. These microorganisms synthesize plant growth promoters, which favor the absorption of such nutrients as N, P, Fe, Zn, Cu. They produce glomalin that binds the soil particles and induce protective action against some soil phytopathogens (Kumar and Verma, 2019).

In the Amancio municipality, the agricultural yields of *G. max* are low and vary, approximately, from 0,9 to 1, t ha$^{-1}$. In recent times, to counteract the negative effect of chemical fertilization the use of biofertilizers is increased, allowing plants to overcome stress situations against adverse conditions of the environment. This favors their growth, development and yield, and contributes to decrease the use of chemical substances.

The objective of this work was to evaluate the effectiveness of the application of three arbuscular mycorrhizal fungi (AMF) strains on the morpho-

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physiological development of *G. max*, cultivar Incasoy 27 on a typical reddish Brown Fersiallitic soil in the Amancio Rodríguez municipality, Las Tunas province, Cuba.

## Materials and Methods

*Location*. The research was developed under field conditions, on a typical reddish-Brown Fersiallitic soil (Hernández-Jiménez *et al*., 2015), in the Cooperative of Credit and Service Mártires de Pino III, Amancio Rodríguez municipality, Las Tunas province, between September and December, 2020. This cooperative is located at the geographical coordinates 24,47′55,1″ North latitude and 77,35 ′ 23,5″ West longitude.

*Soil characteristics in the experimental area*. Samples were taken at 20 cm depth using the experimental grid sampling technique and were dried and sieved with a 2-mm mesh. The pH ($H_2O$) was determined by the potentiometric method, organic matter by Walkley and Black (1934). Cation exchange capacity (CEC), exchange cations ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$) and base exchange capacity (BEC) were determined according to Mehlich (1984), modified by NC-65:2000 (INN and ONN, 2000) (table 1).

*Experimental design and treatments*. The experimental design was a randomized block design with five treatments and four replicas. Plots of 11,2 $m^2$ (2,8 x 4,0 m) were used, with four furrows. Of these, the two central ones (5,6 $m^2$) were taken as the sampling area. The distance between replicas was 1 m and the cultivar INCAsoy-27, from INCA, with 98 % germination, was used. Treatments consisted of an absolute control, NPK and three mycorrhizal strains: INCAM 4, INCAM 11 and INCAM 2.

*Experimental procedure*. The applied crop management was carried out as established by the technical instructions of the crop (Hernández *et al*., 2020). Sowing was carried out in September, 2020, manually at a depth of 4 cm. Two seeds were placed per nest, with a distance between rows of 0,70 m and 0,10 m between plants.

During the crop cycle, seven irrigations were applied using sprinkler technology in the critical periods of water demand, framed in the stages of pre-flowering, flowering-pod formation and grain filling, with an irrigation interval of 7 to 8 days, depending on the rainy period.

NPK was applied at a rate of 10-8-8 at the bottom of the furrow before planting. Arbuscular mycorrhizal fungi strains (*Glomus manioti* sp) were applied as a mixture. The seed was coated two hours before sowing with 2:1 inoculum/water ratio. It was subject to natural drying in the shade.

*Measurements*. Plant height and stem diameter were measured at 30 and 60 days after germination (DAG).

*Statistical analysis*. The variables plant height and diameter at 30 and 60 DAG were statistically processed by a linear model of repeated measures through a full factorial variance analysis, whose assumptions, the normal distribution of data by the Kolmogorov-Smirnov test and the homogeneity of variances by the Levene test were not fulfilled, specifically for the variable plant height at 60 DAG, so the multiple comparison of treatments was performed by Dunnett's (1980) nonparametric T3 test, because the samples per treatment were less than 50 (Shingala and Rajyaguru, 2015).

A Spearman correlation analysis was also performed due to the non-compliance of the normal distribution between the variables plant height and diameter at 30 and 60 DAG, with the aim of establishing the presence or not of relationships between these variables at different plant ages. Automated processing was performed with the SPSS 26 statistical package (IBM, 2019).

## Results and Discussion

*Plant height at 30 and 60 DAG*. Multiple comparison of the treatments (table 2) showed that the control did not differ significantly from the other treatments, except for the treatment in which nitrogen, phosphorus and potassium fertilization was applied. The average height of the control plants was 7,6 cm lower than that of the NPK treatment, with confidence intervals between 5,0 and 10,2 cm lower. In this comparison, the largest confidence intervals were found.

Table 1. Components of soil fertility (0-20 cm).

| Depth | OM | pH | EC | Cmol (+) $kg^{-1}$ | | | | ppm |
|---|---|---|---|---|---|---|---|---|
| cm | % | $H_2O$ | dSm- | $Ca^{2+}$ | $Mg^{2+}$ | $K^{+}$ | $Na^{+}$ | $P_2O_5$ |
| 0-20 | 2,25 | 6,43 | 10,40 | 26,77 | 3,50 | 3,50 | 0,39 | 6,26 |

Table 2. Multiple comparisons of treatment means by repeated measures linear models (30 and 60 days) for plant height.

| (A) Treatment | (B) Treatment | (A-B) | Error ± | Significance | Confidence interval at 95 % | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Higher limit |
| Control | INCAM 11 | -1,687 | 0,6144 | 0,288 | -4,327 | 0,952 |
| | INCAM 2 | 0,038 | 0,5277 | 1,000 | -2,382 | 2,457 |
| | INCAM 4 | -2,850 | 0,6769 | 0,058 | -5,800 | 0,100 |
| | NPK | -7,625* | 0,4829 | 0,001 | -10,161 | -5,089 |
| INCAM 11 | Control | 1,688 | 0,6144 | 0,288 | -0,952 | 4,327 |
| | INCAM 2 | 1,725 | 0,5258 | 0,183 | -0,682 | 4,132 |
| | INCAM 4 | -1,163 | 0,6754 | 0,772 | -4,109 | 1,784 |
| | NPK | -5,938* | 0,4808 | 0,002 | -8,457 | -3,418 |
| INCAM 2 | Control | -0,038 | 0,5277 | 1,000 | -2,457 | 2,382 |
| | INCAM 11 | -1,725 | 0,5258 | 0,183 | -4,132 | 0,682 |
| | INCAM 4 | -2,888 | 0,5977 | 0,052 | -5,804 | 0,029 |
| | NPK | -7,663* | 0,3636 | 0,000 | -9,318 | -6,007 |
| INCAM 4 | Control | 2,850 | 0,6769 | 0,058 | -0,100 | 5,800 |
| | INCAM 11 | 1,163 | 0,6754 | 0,772 | -1,784 | 4,109 |
| | INCAM 2 | 2,888 | 0,5977 | 0,052 | -0,029 | 5,804 |
| | NPK | -4,775* | 0,5586 | 0,011 | -7,916 | -1,634 |
| NPK | Control | 7,625* | 0,4829 | 0,001 | 5,089 | 10,161 |
| | INCAM 11 | 5,938* | 0,4808 | 0,002 | 3,418 | 8,457 |
| | INCAM 2 | 7,663* | 0,3636 | 0,000 | 6,007 | 9,318 |
| | INCAM 4 | 4,775* | 0,5586 | 0,011 | 1,634 | 7,916 |

P- values < 0,05 indicate significant differences between treatments using Dunnett's T3 test for heterogeneous variances.

In the other treatments, INCAM 11, INCAM 2 and INCAM 4, as in the control, the average heights of the plants did not differ statistically from the treatment in which NPK fertilization was applied, that is, the plants fertilized with nitrogen, phosphorus and potassium significantly surpassed the other treatments on average 30 and 60 days after germination as a whole.

The positive effect of AMF on agricultural yields is widely recognized. Corbera-Gorotiza and Nápoles-García (2023) state that mycorrhizae improve the capacity for absorption of water and nutrients from the soil, since their hyphae, by exploring the soil, reach places where it is difficult for plant roots to reach on their own. In addition, AMF increase the hydraulic conductivity of the roots and favor the adaptation of the osmotic balance.

*Stem diameter at 30 and 60 DDG.* The multiple comparison of treatment means by repeated measures linear models for the variable stem diameter (table 3) showed that the control was significantly outperformed by INCAM 11, INCAM 2 and by fertilization with nitrogen, phosphorus and potassium, with average values of 1,13; 0,83 and 0,70 cm, respectively.

In the variable stem diameter, there were significant differences between treatments. The *G. max* plants with the largest diameters corresponded to the treatments with NPK fertilization and with the mycorrhizal strain INCAM 11, without significant differences between them, and with average diameters above four centimeters. The treatments with mycorrhizae INCAM 2 and INCAM 4, with no significant differences between them, achieved average diameters close to 3,5 cm; while the control plants were significantly surpassed by the other treatments with an average diameter of approximately 2,5 cm.

On the other hand, the three treatments with mycorrhizal strains did not differ statistically from

Table 3. Multiple comparisons of treatment means by repeated measures linear models (30 and 60 days) for stem diameter.

| (A) Treatment | (B) Treatment | (A-B) | Error ± | Significance | Confidence interval at 95 % | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Higher limit |
| Control | INCAM 11 | -1,125* | 0,0907 | 0,000 | -1,5272 | -0,7228 |
| | INCAM 2 | -0,687* | 0,0986 | 0,004 | -1,1122 | -0,2628 |
| | INCAM 4 | -0,375 | ,1131 | 0,158 | -0,8704 | 0,1204 |
| | NPK | -0,837* | 0,12479 | 0,008 | -1,4047 | -0,02703 |
| INCAM 11 | Control | 1,125* | 0,0907 | 0,000 | 0,7228 | 1,5272 |
| | INCAM 2 | 0,437* | 0,0875 | 0,027 | 0,0544 | 0,8206 |
| | INCAM 4 | 0,750* | 0,1035 | 0,007 | 0,2626 | 1,2374 |
| | NPK | 0,2875 | 0,1161 | 0,462 | -0,2920 | 0,8670 |
| INCAM 2 | Control | 0,687* | 0,098 | 0,004 | 0,2628 | 1,1122 |
| | INCAM 11 | -0,437* | 0,0875 | 0,027 | 0-,8206 | -0,0544 |
| | INCAM 4 | 0,312 | 0,1106 | 0,280 | 0-,1777 | 0,8027 |
| | NPK | -0,150 | 0,1224 | 0,959 | -0,7172 | 0,4172 |
| INCAM 4 | Control | 0,375 | 0,1131 | 0,158 | -0,1204 | 0,8704 |
| | INCAM 11 | -0,750* | 0,1035 | 0,007 | -1,2374 | -0,02626 |
| | INCAM 2 | -0,312 | 0,1106 | 0,280 | -0,8027 | 0,1777 |
| | NPK | -0,462 | 0,1344 | 0,134 | -1,0466 | 0,1216 |
| NPK | Control | 0,837* | 0,1247 | 0,008 | 0,2703 | 1,4047 |
| | INCAM 11 | -0,287 | 0,1161 | 0,462 | 0-,8670 | 0,2920 |
| | INCAM 2 | 0,150 | 0,1224 | 0,959 | -0,4172 | 0,7172 |
| | INCAM 4 | 0,462 | 0,1344 | 0,134 | -0,1216 | 1,0466 |

Values of $p < 0,05$ indicate significant differences among the treatments with the application of Dunnett's T3 test for heterogeneous variances

the NPK fertilization treatment; values were slightly higher in the INCAM 11 and INCAM 2 treatments. The control did not differ significantly at that age from the INCAM 2, INCAM 4 and NPK fertilization treatments. The only treatment that significantly outperformed the control was the application of mycorrhiza with the strain INCAM 11.

Rodriguez (2019), when analyzing the plant diameter of *G. max* in the Amancio municipality, obtained a performance of 5-7 mm, which coincides with the results of this research. Also Cedeño (2019) evaluated a cultivar of *G. max* under chemical fertilization and inoculated with three strains of EcoMic® and obtained similar results to those of this research in stem diameter, which varied between 5,44-7,60 mm.

The highest confidence intervals among the comparisons of the treatments appeared when comparing the control with INCAM 11. For the variable stem diameter, the confidence intervals at 60 days, after germination for each of the treatments, were widened with regards to the intervals in the evaluations carried out at 30 DAG. This shows increase of the phenotypic variability of that trait in the plants of each of the treatments at that age of the crop.

The improvement in the variables height and diameter of *G. max* plants, which were inoculated with the arbuscular fungi strains, can be consequence of the effects caused by this micorrhizal symbiosis. This is due to the fact that at present the combination of physical, chemical and biological effects on the soil is experimentally acknowledged, which favor the development of the vegetative parts of the plants due to improvements in the processes that regulate and modulate water and nutrient absorption (Igiehon *et al.*, 2021).

It is important that grain-producing plants such as soybean, which constitute their commercial product, reach adequate diameter, aspect that induces strength and resistance to stem lodging in case of occurrence of strong winds and mechanical damage, and that on the contrary if their stems are weak, this can cause drop towards the soil and increase harvest losses because of grain dispersal.

Díaz-Franco *et al.* (2021), in the Río Bravo Experimental Field zone, INIFAP. Tamaulipas, Mexico, obtained higher results in plant height, which varied between 75 and 83 cm, when combining AMF with chemical fertilization.

Data analysis (table 4) proved that 30 days after germination there was phenotypic correlation between height and diameter of the plants, where the values of all treatments were included. Showing significant differences (p < 0,05) such correlation was positive and from moderate to high magnitude, for which it can be interpreted that at 30 DAG as the plant grows in height, stem diameter equally increases.

This direct and positive relation was not observed when the age of the plants reached 60 DAG, because there was no statistical significance between those two variables. Seemingly, as at that age of the crop, plants have slowed their growth to give way to other phases of the crop cycle like the beginning of flowering.

In this sense, to produce *G. max*, in most producing regions worldwide, it is necessary that special attention is paid to crop nutrition, because the productive response that is expected to be achieved depends on it to a large extent. Nutrition has repercussion on harvest quality and influences height from the soil base to the first pod, the weight of one thousand grains and yield (Duran-Mera *et al*., 2021).

## Conclusions

The application of arbuscular micorrhizal fungi strains positively influenced the morpho-physiological indicators evaluated in *G. max*, which allows to decrease the use of mineral fertilizers on this crop.

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## Conflict of interests

There is no conflict of interests among the authors.

## Authors' contribution

- Aracelis Romero-Arias. Research conception and design, data acquisition and interpretation, manuscript writing and revision.
- Sergio Rodríguez Rodríguez. Research conception and design, data acquisition and interpretation, manuscript writing and revision.
- Raquel María Ruz-Reyes. Research conception and design, data acquisition and interpretation, manuscript writing and revision.

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Table 4. Correlation coefficients by Spearman and probability value between plant height and stem diameter 30 and 60 days after germination (DAG).

| | | |
|---|---|---|
| At 30 DAG | | |
| | Plant height, 30 DAG | Stem diameter, 30 DAG |
| Plant height, 30 DAG | - | p = 0.0043 |
| Stem diameter, 30 DAG | r = 0,61 | - |
| At 60 DAG | | |
| | Plant height, 60 DAG | Stem diameter, 60 DAG |
| Plant height, 60 DAG | - | p = 0,81 |
| Stem diameter, 60 DAG | r = - 0,06 | - |

Values of p < 0,05 indicate significant differences in the analysis of correlations between variables

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